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Basement membrane and tumor invasion: ultrastructural observations in the basement membrane of rat bladder with invasive transitional cell carcinoma induced by *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine

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Abstract This study describes ultrastructural alterations in the basement membrane (BM) of rat bladder with invasive transitional cell carcinoma (TCC) induced by *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine. Various alterations including thickening, degradation and neosynthesis were found in the bladder BM of one rat with invasive TCC. Focal destruction of both the BM lamina zones was found in addition to partially degraded BMs showing focal degradation and loss of only the BM lamina rara. Neosynthesis of complete BM including the lamina rara and lamina densa was observed surrounding the nests of carcinoma cells deep in the stroma, while neosynthesis of incomplete BM including only a lamina densa-like structure was also found around carcinoma cells which had just crossed the BM into the adjacent stroma from the original tumor masses. There was an increased hemidesmosomal frequency in some areas of thickened BM, and focal loss of hemidesmosome in the areas of degraded BM. It is suggested that BM degradation may take place in two steps, and that BM neosynthesis may also be a two-step process in invasive TCC of rat bladder.

Key words Basement membrane · Tumor invasion · Transitional cell carcinoma · Degradation · Neosynthesis · Hemidesmosome

Basement membrane (BM) comprises a ubiquitous extracellular matrix found at the boundary of the epithelial cells and connective tissue stroma. At the level of pathological anatomy, the BM can be understood as the structural barrier to the invasion and metastatic behavior of malignant cells. Tumor invasion can be defined as the active migration of neoplastic cells out of their tissue of origin and into different types of adjacent tissue. During the transition from in situ to invasive carcinoma, tumor cells penetrate the epithelial BM and enter the underlying interstitial stroma [17]. As a general feature of all types of carcinoma [1], defects in the BM are associated with the tumor invasion [15]. Although the loss of BM in invasive carcinoma has received much attention, several ultrastructural studies have demonstrated tumor invasion in the presence of an essentially complete BM [5, 8] and a thickened BM [6]. *N*-Butyl-*N*-(4-hydroxybutyl)-nitrosamine (BBN) has been proved to be an effective organ-specific carcinogen on urinary bladder of rat, and is widely used in the study of bladder tumor in animal models [13, 14]. This study reports various changes in the BM of rat bladder with invasive TCC induced by administration of BBN for 28 weeks using transmission electron microscopy (TEM).

Materials and methods

Twenty male Wistar rats (Kurea, Osaka, Japan), 7 weeks of age at the start of the experiment were divided into a BBN group (15 rats) and a control group (5 rats). Rats were housed three to a polycarbonate cage, placed in an environmentally controlled room illuminated for 12 h/day. BBN (Kasei, Tokyo, Japan) was administered at 0.05% in drinking water for 28 weeks in the BBN group. All 15 rats in the BBN group and the 5 rats in the control group were put to death 28 weeks from the start of the experiment. The specimens of rat bladder obtained were examined by light microscopy and TEM, respectively. For light microscopy one part of the bladder was placed in 10% formalin and processed for paraffin embedding. Each paraffin block was step-sectioned and stained with hematoxylin and eosin. Multiple sections of each bladder were examined. For TEM observation the specimens were cut into 4×4×4-mm blocks and fixed with 2.5% glutaraldehyde for 4 h at room temperature. Following a phosphate buffer wash, the specimens were post-fixed in 1% osmic acid for 2 h, then dehydrated in graded ethanols, treated with propylene oxide and embedded in Epon 812. Ultrathin sections were cut on a LKB ultramicrotome with a diamond knife and examined with a Hitachi JEM-1200 EX TEM.

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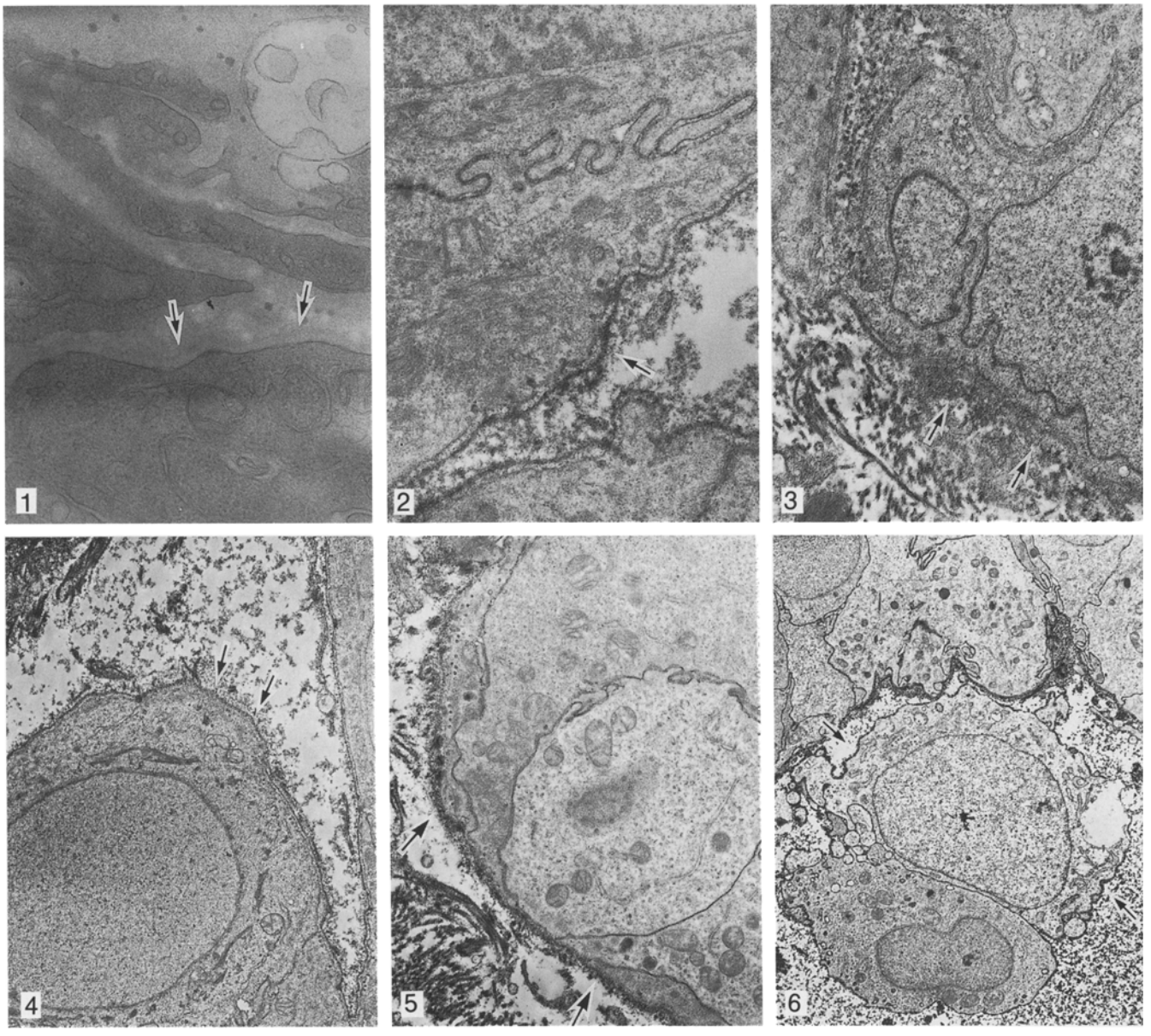

Fig. 1 The BM is smoothly arranged at the junction of the basal epithelia and interstitial stroma. Two distinct BM zones, the lamina rara and the lamina densa, are of the same thickness (→). The hemidesmosomes are also observed on the plasma membrane of basal cells. Control group, 28 weeks, TEM, ×20000

Fig. 2 Focal thickening in the BM (→) of rat bladder with invasive TCC. The lamina rara is thickened to the same extent as the lamina densa. BBN group, 28 weeks, TEM, ×15000

Fig. 3 Focal destruction is observed in the BM of rat bladder with invasive TCC. Both BM lamina zones are degraded (→). BBN group, 28 weeks, TEM, ×8000

Fig. 4 Partially degraded BM and focal degradation of the lamina rara in the BM (→) of rat bladder with invasive TCC. An increased hemidesmosomal frequency is observed in the thickened BM area. BBN group, 28 weeks, TEM, ×5000

Fig. 5 Partially degraded BM, showing focal loss of only the BM lamina rara, is found in the BM of rat bladder with invasive TCC. The carcinoma cells are covered only by the BM lamina densa (→). BBN group, 28 weeks, TEM, ×6000

Fig. 6 Neosynthesis of incomplete BM with only a lamina densa-like structure (→) is found surrounding the tumor cells which have just crossed the BM into the stroma from the original tumor masses. BBN group, 28 weeks, TEM, ×3000

Results

Light microscopic findings

In the control group the rat bladder epithelium appeared normal in all five rats. In the BBN group invasive TCC of the bladder was observed in all 15 rats. The bladder tumor had focally invaded into the muscle layers, but had not gone beyond the rat bladder wall.

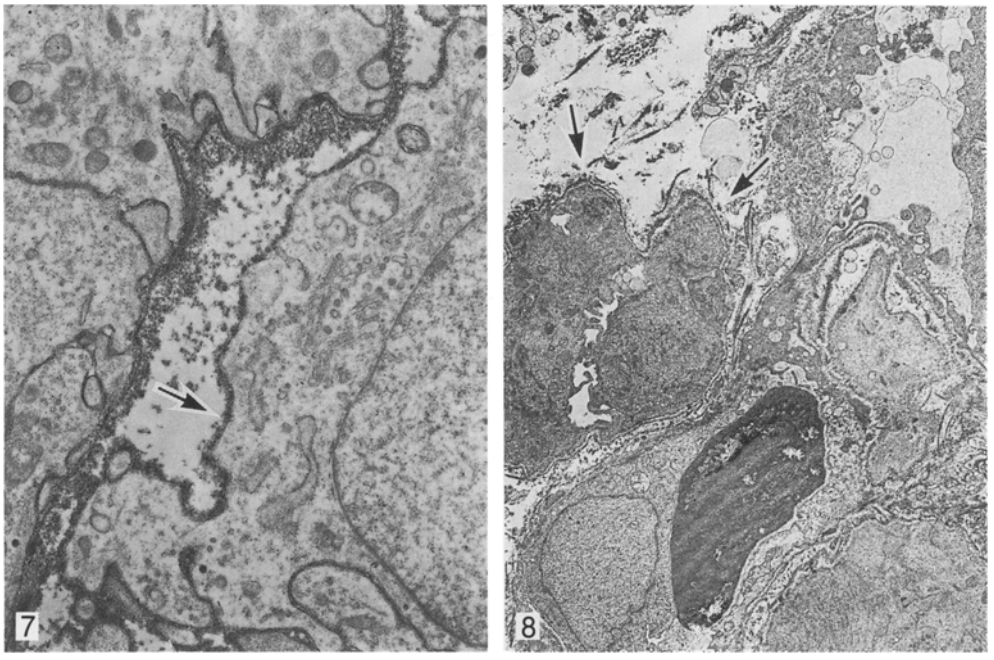

Fig. 7 Higher magnification of Fig. 6, ×8000

Fig. 8 Neosynthesis of complete BM including both BM lamina zones (→) around the nests of tumor cells deep in the stroma. BBN group, 28 weeks, TEM, ×3000

TEM findings

In the control group the continuous BM was smoothly arranged at the junction of the basal epithelia and interstitial stroma. The lamina rara and lamina densa, the two distinct zones of the BM, were of the same thickness (Fig. 1). The hemidesmosomes connecting the basal cells to the BM were observed on the plasma membrane of basal cells adjacent to the BM. In the BBN group various alterations were observed in the BM of rat bladder with invasive TCC.

Focal thickening and loss in the BM

Focal thickening and loss in the BM were observed in all 15 rats with invasive TCC. Both thickened and degraded BM could be observed just in one rat bladder, although in different parts. In the area of thickened BM the lamina rara was thickened to the same extent as the lamina densa (Fig. 2). In the areas of degraded BM, focal destruction of the two BM lamina zones was found as well as focal degradation and loss of only the BM lamina rara. With focal destruction there was no BM at the junction of the tumor cells and the adjacent stroma (Fig. 3). With focal degradation the BM was partially degraded, with only the lamina densa remaining at the junction of the tumor cells and the adjacent stroma (Figs. 4, 5).

Neosynthesis of BM components

Neosynthesis of BM components was found surrounding the carcinoma cell islets invading the interstitial stroma. Formation of an incomplete BM of only a lamina densa-like structure was found surrounding the carcinoma cells which had just crossed the BM into the adjacent stroma from the original tumor masses (Figs. 6, 7), while the formation of complete BM including both the lamina rara and lamina densa was also found around the nests of carcinoma cells deep in the interstitial stroma (Fig. 8).

Alterations in hemidesmosomal frequency

Focal loss of hemidesmosome was accompanied by tumor invasion and BM degradation (Fig. 3). An increased hemidesmosomal frequency was observed in some areas of thickened BM (Fig. 4).

Discussion

With its function of separating the epithelial and other cells from the surrounding stroma, the BM presents an obstacle for invading tumor cells [1]. Consequently the process of tumor invasion and metastasis is characterized by loss of BM. This loss and destruction of BM is accompanied by an increased release of proteolytic enzymes that digest BM and decreased formation of BM [17]. It is believed that the tumor cells directly secrete degradative enzymes or induce the host to produce proteolytic enzymes to degrade the BM [15]. Consequently, at the point of invasion the BM is usually discontinuous [4, 12]. The results of this study also show that tumor invasion is accompanied by BM degradation in invasive TCC of rat bladder induced by the administration of BBN.

Some authors have reported that focal defects in the continuity of the BM lamina densa can be found in in situ

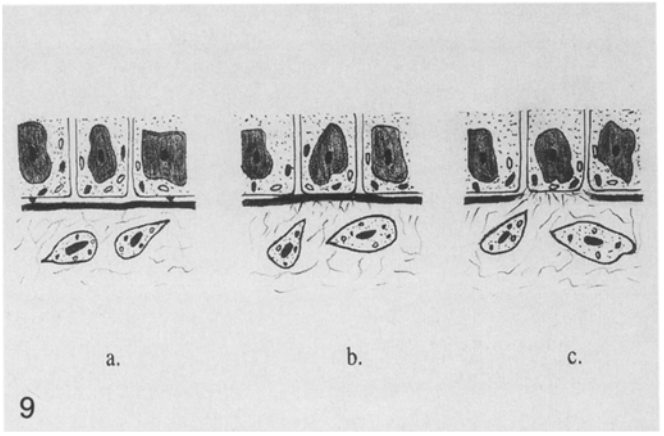

Fig. 9a–c The two steps of BM degradation. **a** Thickened BM of rat bladder with invasive TCC. **b** Partially degraded BM, representing focal loss of only the BM lamina rara. **c** Focal loss of both BM lamina zones

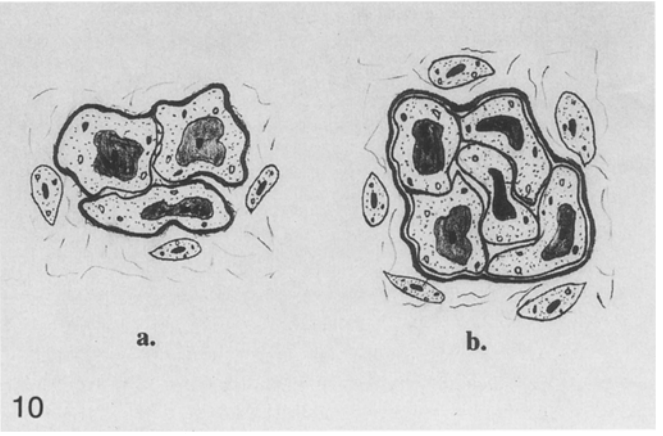

Fig. 10a, b The two steps of BM neosynthesis. **a** Formation of incomplete BM with only a lamina densa-line structure surrounding the carcinoma cells which have just crossed the BM into the adjacent stroma from the original tumor masses. **b** Formation of complete BM including both BM lamina zones around the nests of carcinoma cells deep in the interstitial stroma

carcinoma lesions, and that these defects can be regarded as the earliest stages of progression to invasive carcinoma [17]. However, it is worth noting that in our study both destruction of two BM distinct zones and degradation of only BM lamina rara were observed, indicating that the degradation of lamina rara may occur earlier than the degradation of lamina densa in the process of tumor invasion. Based on these findings, it is suggested that the degradative process of BM in the invasive TCC of rat bladder may be in two steps, and the loss of the BM lamina rara may be regarded as the first step of BM degradation (Fig. 9). It is also indicated that the loss of the lamina rara may be induced by an increased release of degradative enzymes directly secreted from the carcinoma cells on the lamina rara while the loss of the lamina densa may be due to an increased release of proteolytic enzymes secreted both by the carcinoma cells and by mesenchymal cells. Of course, decreased synthesis of BM components also plays an important role in the degradation of the BM lamina rara and lamina densa.

The epithelial cells not only degrade, but also produce, their own BM and may continue doing so after malignant transformation [16]. Previous studies also reported several continuous BMs and thickened BMs in invasive carcinoma [5, 6, 8]. It is well known that one of the major characteristics of invasive carcinoma is its behavior of invasion and metastasis, but it is also crucial to remember that the invasive tumor cells are characterized by the highest cellular metabolic activity. Thus, the invasive tumor cells may release large amounts of secretory product of cellular metabolism that are believed to be the necessary components of a BM. In this study focal thickening in the BM of rat bladder with invasive TCC was frequently observed, and the lamina rara was thickened to the same extent as the lamina densa. It is suggested that the thickened BM in invasive TCC of rat bladder may result from increased synthesis of BM components, and the occurrence of thickened BM in invasive carcinoma can be expected, whether degradation and loss in the BM can be found or not.

In previous studies, neosynthesis of BM components was found surrounding the tumor cell islets in cases of invasion [9, 10]. On the other hand, some authors reported that normal epithelial and benign tumor cells may require a BM for attachment and growth [20]; in contrast the invasive tumor cells may not have this requirement [1]. In the present study neosynthesis of complete BM was found around the nests of carcinoma cells deep in the stroma, while neosynthesis of incomplete BM including only the lamina densa-like structure was also found surrounding the carcinoma cells which had just crossed the BM into the adjacent stroma from the original tumor masses. It means that formation of the lamina densa may occur earlier than formation of the lamina rara in neosynthesis of BM. These findings suggest that neosynthesis of BM in the invasive TCC of rat bladder may also be in two steps (Fig. 10), and neosynthesis of the BM lamina rara may be carried out by carcinoma cells on the lamina rara, while neosynthesis of the BM lamina densa may be carried out both by carcinoma cells and by mesenchymal cells. The difference in localization between complete BM and incomplete BM may be explained as follows:

1. Before penetrating the BM and into the adjacent stroma, the carcinoma cells must dissolve and destroy the BM. It therefore appears logical that the carcinoma cells cannot possess a complete BM when they have just crossed the BM into the adjacent stroma from the original tumor masses.
2. The carcinoma cells that have invaded the stroma may require a complete BM for attachment and growth. This requirement has resulted in the formation of a complete BM around the tumor cell islets deep in the stroma due to an increased secretion of BM components both by carcinoma cells and by mesenchymal cells.

In the present study, although the ultrastructural observations showed various alterations, including thickening, degradation and neosynthesis as mentioned above, in the BM of rat bladder in a similar case, the histological findings displayed invasive TCC of bladder in all 15 rats following the administration of BBN for 28 weeks. The reason for this phenomenon may be the dual properties shown by the carcinoma cells. One property is to degrade and penetrate through the BM into the adjacent stroma tissues through an increased release of degradative enzymes. The other property is to synthesize the BM by means of increased secretion of the BM components. Of course, the mesenchymal cells may play the major role in both the degradation and neosynthesis of BM.

The function of the hemidesmosomes is thought to be adhesion between the epithelial cells and their basal lamina. In previous studies many authors have reported decreased hemidesmosomal frequency in the malignant lesions [11, 18, 19]. The decreased number of hemidesmosomes may result from failure of hemidesmosomal formation or from increased hemidesmosomal degradation [19]. In this study the focal loss of hemidesmosomes was accompanied by BM degradation, while and increased hemidesmosomal frequency could be found in some areas of thickened BM. Based on these findings it is suggested that focal loss of hemidesmosomes may result from increased hemidesmosomal degradation in the invasive TCC. The increased hemidesmosomal frequency in the thickened BM area may be considered the result of increased hemidesmosomal formation, because hemidesmosome frequency is positively correlated with cell proliferation [7]. It has been reported that the reductions in hemidesmosomal frequency could reflect an increased mobility of basal epithelial cells and a decreased adherence of basal cells to the adjacent basal lamina [19]. Thus, tumor-invasive processes may also be characterized by hemidesmosomal degradation.

References

1. Barsky SH, Siegal GP, Jannotta F, Liotta LA (1983) Loss of basement membrane components by invasive tumors but not by their benign counterparts. Lab Invest 49:140
2. Birembant P, Caron Y, Adnet JJ, Foidart JM (1985) Usefulness of basement membrane markers in tumoral pathology. J Pathol 145:283
3. Bosman FT, Havenith M, Cleutjens JPM (1985) Basement membranes in cancer. Ultrastruct Pathol 8:291
4. Burtin P, Chavanel G, Foidart JM, Martin E (1982) Antigens of the basement membrane and the peritumoral stroma in human colonic adenocarcinoma: an immunofluorescence study. Int J Cancer 30:13
5. Carter RL, Burman JF, Barr L, Gusterson BA (1985) Immunohistochemical localization of basement membrane type IV collagen in invasive and metastatic squamous carcinoma of the head and neck. J Pathol 147:159
6. Chomette G, Auriol M, Tranbaloc P, Blondon J (1990) Stromal changes in early invasive breast carcinoma – an immunohistochemical, histoenzymological and ultrastructural study. Pathol Res Pract 186:70
7. Christophers E, Wolff HH (1975) Differential formation of desmosomes and hemidesmosomes in epidermal cell cultures treated with retinoic acid. Nature 256:209
8. Dingemans KP (1988) What's new in the ultrastructure of tumor invasion in vivo. Pathol Res Pract 183:792
9. Forster SJ, Talbot IC, Critchley DR (1984) Lamina and fibronectin in rectal adenocarcinoma: relationship to tumor grade, stage and metastasis. Br J Cancer 50:51
10. Gusterson BA, Clinton SS, Gough G (1986) Studies of early invasive and intraepithelial squamous cell carcinoma using an antibody to type IV collagen. Histopathology 10:161
11. Halter SA, Glick AD (1983) Basement membrane changes in human breast tissue in explant culture: a morphometric and ultrastructural study. Ultrastruct Pathol 4:23
12. Harvenith MG, Arends JW, Simon REM, Volovics A, Wiqqers T, Bosman FT (1988) Type IV collagen immunoreactivity in colorectal cancer: prognostic value of basement membrane deposition. Cancer 62:2207
13. Ito N (1976) Early changes caused by *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine in the bladder epithelium of different animal species. Cancer Res 36:2528
14. Ito N, Hiasa Y, Okajima E, Kitamura H (1969) Histogenesis of urinary bladder tumors induced by *N*-butyl-*N*-(4-hydroxybutyl)-nitrosamine. Gann 60:401
15. Liotta LA, Stetler-Stevenson WG (1991) Tumor invasion and metastasis: an imbalance of positive and negatie regulation. Cancer Res 51:5054
16. Liotta LA, Foidart JM, Gehron-Robey P, Martin GR, Gullino PM (1979) Identification of micrometastasis of breast carcinomas by presence of basement membrane collagen. Lancet II:146
17. Liotta LA, Rao CN, Barsky SH (1983) Tumor invasion and the extracellular matrix. Lab Invest 49:636
18. White FH, Gohari K (1981) A quantitative study of lamina densa alterations in hamster cheek pouch carcinogenesis. J Pathol 135:277
19. White FH, Gohari K (1984) Hemidesmosomal dimensions and frequency in experimental oral carcinogenesis: a stereological investigation. Virchows Arch [B] 45:1
20. Wicha MS, Liotta LA, Vonderhaar B, Lewko WM, Kidwell WR (1979) Effect of blocking basement membrane collagen synthesis on the growth of normal and neoplastic mammary cells in vivo and in vitro (abstract). J Cell Biol 83:58a